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Demographic Parameters and Twinning: A Study in Catalonia, Spain

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Abstract. Twinning rates for the years 1975-79 in Catalonia (Spain) are presented. Crude rates are very low: 7.62 per 1,000 maternities, the DZTR and the MZTR being 3.74 and 3.88 respectively. Standardized rates remain very low. Sex ratio among twin couples is also very low (0.49 male vs 0.51 female births). A multiple linear stepwise regression on the twinning rates shows MZ rates to be influenced by birth order and father's age, and the DZ rates by mother's age and birth order.

Key words: Twinning rate, Father's age, Mother's age, Birth order

INTRODUCTION

The incidence of twinning varies considerably in human populations, the major changes relating to the dizygotic component [3].

Among the factors affecting twinning, the influence of maternal age and parity is well known [2] and there are some insights on the effect of nutritional [14] and environmental [4,12] factors, as well as psychosocial influences [16,21], while the role played by other factors is less clear. The genetic component of twinning has been investigated, among the others, by Parisi et al [17] who found that twinning proneness can be inherited, mainly through the maternal line.

Studies on multiple births in Spain have been quite few. In a pioneering work, Bulmer [3] reported a twinning rate (TR) for the years 1951-1953 of 9.1 per 1,000 maternities, the dizygotic twinning rate (DZTR) being 5.9, and the monozygotic twinning rate (MZTR) 3.2. These rates were the lowest reported for European populations. Low rates in Spain were also reported for the years 1951-1967 by Valls [22], the crude rate being 9.5, DZTR = 6.0 and MZTR = 3.5, per 1,000

maternities. In a recent work [1] we have examined data for the years 1975-1979 (there are no available data for the years 1968-1974) finding crude rates of 8.44, $DZTR = 4.17$ and $MZTR = 4.27$, per 1,000 maternities. After standardization by the direct method [6] using the proportion of mother's age groups for twinning births in Spain in 1951-53 reported by Bulmer [3], the TR was 8.87 per 1,000 maternities.

Although available data are too limited in scope to draw conclusions on the trend of twinning in Spain, the figures reported here are in agreement with the long-term decline in twinning rates observed in a number of countries. This decline is due exclusively to a fall in the $DZTR$, and has proved to be of difficult interpretation [5,9,12,15,16].

In this work we present data on twinning for years 1975-1979 in Catalonia, in the Northeast of Iberia with four provinces (Barcelona, Girona, Lleida and Tarragona) and a population around 6 million inhabitants (16% of the whole of Spain). Together with a general description we have performed an analysis of multiple regression to assess the effects of parental age and birth order on twinning rates.

MATERIALS AND METHODS

All the information on births, stillbirths and deaths occurring in Spain is collected by the *Instituto Nacional de Estadística*, which publishes global figures. Some of them are of interest in the study of twinning and have been used in early works [3,22], but they offer time-changing information which is only on sex of newborns, province of birth and recently on life-status. Those variables are considered one by one, so the analysis has to be very superficial.

The data used in this study are much more complete than those given in official publications and were recorded on tapes, which contained all the information related to the 502,469 births occurring in Catalonia between 1975 and 1979. This information was gathered by the *Instituto Nacional de Estadística*, and made available to us through the *Conselleria de Sanitat de la Generalitat de Catalunya*. For each birth, 32 variables are considered. We mainly use, in this work, parental age, birth order, sex and multiplicity.

From the general files we built new files with only twins. When trying to match them in couples, some were not found because one of the newborns was a stillbirth. We looked for them in stillbirth files in order to have all them in couples with complete data. Statistical analyses were carried out at the Informatic Center of the University of Barcelona.

A multiple regression was used in order to examine the possible association between parental ages and birth order simultaneously with twinning rates. First of all, the three factors were divided into classes; the number of classes per factor was as follows:

<i>Variable</i>	<i>Classes</i>
Maternal age	<20, 20-, 25-, 30-, 35-, 40+
Paternal age	<20, 20-, 25-, 30-, 35-, 40-, 45+
Parity	1, 2, 3, 4, 5, 6, 7, 8, 9+

Next, we built a series of cells, each of them being a unique combination of the classes of the three variables and containing the number of births according to multiplicity. The number of cells thus obtained was 378 ($6 \times 7 \times 9$). The information contained in each cell was: total births and twin deliveries according to sex of newborns. Cells containing any zero value were excluded from the analysis; thus, the total number of non-empty cells was 178.

The independent variables of the regression were parental ages and parity, the TR, DZTR and MZTR being the dependent variables. The parameters of the equation were estimated by the least-squares method, using a weighting factor, which was taken as the inverse of the variance of the calculated rate:

$$wf = N_c/p_cq_c$$

where N_c = total births of cell c , p_c = rate of cell c (which was for the different analyses the TR, DZTR and MZTR for each cell) and $q_c = 1 - p_c$.

The use of such a factor usually generates through the calculations a high number of cases, which is, of course, unreal. Therefore, a correction was made so that the final number of cases was the number of non-empty cells (178).

The regression was carried out using the program REGRESSION of the SPSSX statistical package. This program performs a stepwise regression, which consists of the construction of an equation with the least possible number of terms. The independent variables enter one at a time, and only if they reach certain statistical criteria. The order of inclusion depends on the respective contribution of each variable to the explained variance of the dependent variable. That is, at each step the analysis includes in the equation the variable that accounts for the greatest percentage of the variance of the rate which is not explained by the variables already entered in the equation.

RESULTS AND DISCUSSION

Incidence of Twinning

The total number of maternities recorded for years 1975-1979 in Catalonia was 498,586, with 3,803 pairs of twins and 40 sets of triplets. No higher multiples were recorded. Using the difference method of Weinberg, the crude twinning rate was 7.62 per 1,000 maternities, the DZTR and MZTR being 3.74 and 3.88, respectively. After standardization by the direct method using the proportions of mother's age groups for twin births in Spain for the years 1951-1953, the general twinning rate

was 8.08 per 1,000 maternities, the DZTR being 4.17 and the MZTR 3.91. Over the studied period the rates of twinning remained fairly constant. Catalonia shows one of the lowest rates in Spain. Although no clear pattern can be observed on the variation of twinning rates in Spain, low values are found in the North, fact already reported for the 1950s by Bulmer [3].

It is generally accepted that the sex ratio among twin births is lower than in singletons [4,8,10-13]. In our data the sex ratio among twins was 0.4917, which is significantly lower than the ratio in singleton births for the same period, 0.5166 [19]. In order to estimate the sex ratio by zygosity, it can be assumed that DZ twins have the same ratio as singletons, so that the sex ratio in MZ twins can be estimated at 0.468. This figure is even lower than the estimate of 0.496 reported by James [10], reflecting a great fetal-loss of male-male couples in MZ pregnancies.

Table 1 - Twinning rates by mother's age

	< 20	20-24	25-29	30-34	35-39	40+
Twin maternities	98	836	1393	940	374	141
Twinning rate	4.88	6.45	7.76	9.29	9.18	8.70
MZ rate	3.27	3.90	3.98	3.88	4.26	3.05
DZ rate	1.61	2.55	3.78	5.41	4.92	5.65

Table 2 - Twinning rates by father's age *

	< 20	20-24	25-29	30-34	35-39	40-44	45+
Twin maternities	24	383	1325	1037	511	258	87
Twinning rate	4.91	6.16	7.23	8.05	9.10	9.17	9.11
MZ rate	2.86	4.00	3.84	3.95	3.94	4.05	3.46
DZ rate	2.05	2.16	3.39	4.10	5.16	5.12	5.65

* There are 157 cases of father's age missing because of illegitimacy.

Table 3 - Twinning rates by birth order

	1	2	3	4	5	6+
Twin maternities	1299	1185	700	319	138	141
Twinning rate	6.14	7.18	9.51	11.97	13.30	16.96
MZ rate	3.18	3.68	4.81	6.13	5.72	7.94
DZ rate	2.96	3.50	4.70	5.84	7.58	9.02

Analysis of Demographic Factors

The total, MZ and DZ twinning rates are given by mother's age in Table 1, by father's age in Table 2, and by birth order in Table 3.

In our data the twinning rate increases with maternal age up to a peak at 30-34 years and then falls; the increase is mainly due to variation in the DZTR. The mean of the maternal age distribution for twin births (28.415 ± 0.087) was significantly higher than for singletons (27.239 ± 0.008). The mean was of the same order in both types of like-sexed twins (MM = 28.264 ± 0.144 ; FF = 28.056 ± 139), and significantly higher in unlike-sexed twins (MF = 29.215 ± 0.153).

The variation of twinning rates with father's age shows the same general tendency as for mother's age, with a peak at 40-44 years. For both ages, no clear pattern is seen in the MZTR. The DZTR increases in general with age, without the decline in the oldest ages that has been reported in other populations.

When considering the effect of parity, a continuous rise is observed in all twinning rates reaching, for the highest birth order considered, 2.8 times the value for the first delivery. Although the effect is less marked in the MZTR than in the DZTR, both rates show a clear increase with parity.

We have no available data concerning the use of ovulation stimulants which are levelling up twinning in this population, but an indirect indication of such effect could be the relatively high DZTR for primiparae in comparison with the values for mothers aged under 25 (see Tables 1 and 2). In our data 15% of mothers of twins are aged over 30 and still primiparae (10% of total births are to primiparae over 30). Most of these mothers could be led to treatment of infertility. The increased risk of DZ twinning following the use of ovulation stimulants is well known and it has been estimated that 10% of twin births follow the use of these drugs in France [7]. This fact can account in part for the rise (22.5% to 34.2%) observed in unlike-sex twins born to primiparae aged under and over 30, respectively.

Previous studies have reported that the frequency of twin births is positively associated with maternal age independently of parity and viceversa, the overall variation being attributed to DZ twinning, while MZ rates remain fairly constant. In fact, some authors have found a small increase of the MZTR with maternal age [14]. The effect of father's age has not been clearly established but seems to have no important effects.

Since father's age (PA), mother's age (MA) and birth order (BO) are known to be highly correlated (in our sample, the correlation coefficients are PA-MA 0.7994, PA-BO 0.5234 and MA-BO 0.5201), it may be assumed that the pattern shown by any of them is but the reflection of the pattern shown by the others. In order to separate the influence of the two main factors (MA and BO) on the probability of a DZ twin birth, a bivariate analysis has been undertaken.

Table 4 shows the percentages of unlike-sex twins by mother's age and birth order. It is noticeable that we have failed to find the generally accepted effect of birth order on twinning independently of mother's age. This could suggest that parity is not an important factor for DZ twinning, but it could also be due to the size of the sample. Later we will return to this by a statistical method.

Table 4 - Percentage of unlike sex twins by mother's age and birth order

Birth order	< 20	20-24	25-29	30-34	35-39	40 +
1	16.90	18.43	23.59	34.59	26.09	
2	19.05	19.92	22.55	28.25	25.00	
3		16.67	23.66	29.20	18.68	
4			15.19	28.57	27.87	23.53
5+			13.16	31.11	29.00	27.08

As expected, the protogenetic interval (span of time from marriage to labour) is longer for unlike-sex than for like-sex twins (29.36 vs 23.81 months; $t = 2.99$, $P = 0.003$).

Multiple Linear Regression

One of the first aims of this study was to investigate the influence of demographic variables (mother's age, father's age, and birth order) on twinning. The effect of these variables has been the subject of a number of studies; however, most of them have centered the analysis on univariate or bivariate descriptions.

In the foregoing we analyse the effects of the aforementioned variables on twinning by using the multiple linear regression (MLR) model described in the methods section. MLR has proved to be a useful tool in the analysis of problems of similar nature, eg, the influence of demographic factors which influence sex ratio [18].

Table 5 shows, separately for TR, MZTR, and DZTR, the values of the multiple correlation coefficient (R), its standard error (R^2), and the change of R^2 , which represents the contribution added to the explained variance of the rate considered. The results of the analysis of variance are shown to test the significance of the regressions.

When considering the twinning rate (TR) the first variable which enters the regression is birth order, with a high coefficient of correlation ($R = 0.596$). This means that this variable accounts by itself for a 35.5% of the variation of the TR. By adding the two other variables, the R value increases to 0.621 and the percentage explained rises to 38.5%. Only a supplementary 3% of the variation is explained by the new variables, age of the mother and age of the father. Although the regression is significant in both cases, the change in R^2 when adding those two variables is rather negligible when compared to the first one. Due to this low value, the variables are not entered separately.

Table 5 - Order of inclusion of the variables in the regression equation

Variable	R	SE	R ²	R ² change	Analysis of variance		
					F	df	P
<i>Twinning rate</i>							
BO	0.59624	0.00195	0.35550	0.35550	97.079	1;226	0.0000
MA and PA	0.62077	0.00192	0.38536	0.02986	36.364	3;174	0.0000
<i>MZ rate</i>							
BO	0.28043	0.00123	0.07864	0.07864	11.779	1;138	0.0008
PA	0.37877	0.00119	0.14346	0.06482	11.473	2;137	0.0000
MA	0.38292	0.00119	0.14663	0.00317	7.789	3;136	0.0001
<i>DZ rate</i>							
MA	0.57759	0.00129	0.33361	0.33361	58.572	1;117	0.0000
BO	0.61745	0.00125	0.38124	0.04764	35.736	2;116	0.0000
PA	0.62361	0.00125	0.38889	0.00764	24.394	3;115	0.0000

BO = birth order; MA = maternal age; PA = paternal age.

When analysing the MZ rate, the first variable which enters the equation is also the birth order, but with a lower value of R (0.280), and so the percentage of variation explained is only 7.9%. The second variable in importance is the age of the father, which introduces a significant percentage of explanation of the dependent variable (F change = 10.34, P = 0.0016), rising to 14.3%. The third variable, age of mother, rises the multiple correlation coefficient to 0.383 and R² to 0.147, but the change is not significant (F change = 0.50, P = 0.4787) and its effect on the MZ rate must not be considered.

The regression on the DZ rate shows a clearly different pattern. The most explicative variable is now, as extensively reported, the age of the mother with a correlation coefficient of 0.578, and so with a 33.4% of the variation explained. Next in importance is the birth order (R = 0.617, R² = 0.381) with a significant increase in the percentage of explanation (F change = 8.93, P = 0.0034). The third variable, age of the father, should be excluded because of the nonsignificant change in the R² produced (F change = 1.44, P = 0.2328).

All the regressions performed are highly significant, even when introducing variables which produce more noise than explanation. This is due to the good regression obtained when considering the first variable.

Most of these results can also be seen in Table 5, where the coefficients of regression are presented with a significance test for being non-zero values. The importance of birth order in the three regressions and of mother's age in the DZ rate can be clearly seen from the regression coefficients.

Search has been undertaken in order to find some kind of nonlinear relationship that could explain a higher percentage of the variation of the twinning rate. Some functions have been used, such as exponential, potential and logarithmic, and the

same program has been applied. All give lower values than those obtained with untransformed data.

Residual analysis, considering the different weighting factors of each cell-case, has not shown any biased distribution when compared to predicted values.

The MLR analysis shows twinning to be clearly influenced by all three demographic factors (PA, MA and BO) studied in this work. The effects of these variables have to be separately considered for MZ and DZ twinning. Thus, in the case of MZ twinning, BO is the most accounting variable; the effect of PA is also statistically significant but not that of MA. In the case of DZ twinning, the main effect is due to MA; BO also has significant effect, but PA has not. Notably, MLR analysis has shown the effect of BO on DZTR, which is not apparent in the bivariate analysis (Table 6).

Table 6 - Coefficients of regression and their significance tests. For each case a regression is made with the most important variable and with all three.

Model	$b_1(\times 10^{-4})$	T	P	$b_2(\times 10^{-4})$	T	P	$b_3(\times 10^{-4})$	T	P	C($\times 10^{-4}$)
<i>Twinning rate</i>										
BO	13.91	9.8	0.0000							43.52
BO,MA,PA	13.54	7.7	0.0000	5.44	2.7	0.0068	-4.71	2.4	0.0141	45.03
<i>MZ rate</i>										
BO	3.27	3.4	0.0008							28.06
BO,MA,PA	5.88	4.8	0.0000	-1.06	0.7	0.4787	-2.89	2.0	0.0436	36.79
<i>DZ rate</i>										
MA				8.02	7.6	0.0000				7.47
BO,MA,PA	5.19	3.2	0.0018	6.73	4.0	0.0001	-1.95	1.2	0.2328	8.70

MA = maternal age; BO = birth order; PA = paternal age. C = constant.

As it has been shown in a previous study on the sex ratio by MLR analysis [20], the effects of demographic variables measured by regression could vary among populations depending on the genetic and demographic background.

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